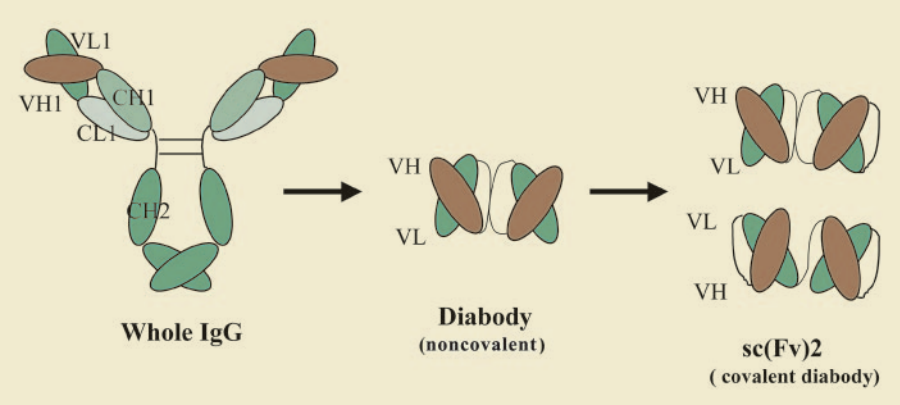

Dramatic conversion of the agonistic activities of VB22B antibody against TPO dependent cells. See the complete figure in the article beginning on page 562.

to alloimmunization. Additional strategies for the treatment of thrombocytopenic patients would be beneficial.

Thrombopoietin (TPO, c-mpl ligand) is a lineage-specific regulator of megakaryopoiesis and platelet production. In humans, 2 recombinant forms of TPO (pegylated recombinant human megakaryocyte growth and development factor [PEG-rhMGDF] and recombinant human TPO [rhTPO]) have been used to treat thrombocytopenia.[2] TPO acts by binding to c-mpl, a type I hematopoietic transmembrane receptor. Efficacy has been demonstrated for TPO in the treatment of thrombocytopenia in patients receiving nonmyeloablative chemotherapy. In contrast, TPO is not effective for patients receiving myeloablative chemotherapeutic regimens. In addition, TPO treatment may be complicated by the production of neutralizing and nonneutralizing antibodies that cross-react with native TPO.

In this issue of *Blood,* Orita and colleagues have taken a novel approach to create alternative c-mpl agonists. They began by identifying c-mpl agonist antibodies, antibodies that activate instead of block receptor function. However, they found that even antibodies with reasonably high affinity displayed weak agonist activity. Hypothesizing that the weak agonism of these antibodies may be due to inefficient receptor dimerization because of steric hindrance, they created 2 types of dimeric antibody fragments, or minibodies, in an attempt to improve c-mpl activation. One of these (diabody) is a noncovalent dimer, while the other (sc(FV)2) is covalent (see figure). Minibodies from 2 antibodies, VB22B and TA136, were characterized.

The first minibody, VB22B sc(Fv)2, increased proliferation of M-07e cells (a TPO-dependent, megakaryocytic cell line) as well as CD41$^+$ cells from a population of human bone marrow progenitor cells. This minibody displayed potency similar to TPO. VB22B sc(Fv)2 was also shown to have in vivo activity in normal cynomolgus monkeys. Although increased platelet counts were seen in monkeys treated with either VB22B sc(Fv)2 or TPO, rebound thrombocytopenia was seen only with TPO. Although relatively few animals were used, these experiments are exciting, and support the performance of more extensive studies in vivo of this novel c-mpl agonist.

Interestingly, TA136 sc(Fv)2, another minibody, was found to increase proliferation of TPO-dependent cell lines derived from 2 patients with congenital amegakaryocytic thrombocytopenia (CAMT), a disorder caused by mutant c-mpl. The agonist activity of TA136 sc(Fv)2 was greatly improved over TPO at these mutant receptors. This suggests a paradigm in which antibody fragments could be designed to treat a variety of receptor-based diseases.

One implication of the work by Orita et al, is that alternative c-mpl agonists may represent a new approach to the treatment of thrombocytopenia. However, another interesting aspect of this work is the creation and use of the minibodies themselves. Minibodies could be created against a variety of growth and differentiation receptors that require dimerization for activation. Creation of such molecules could be useful in the treatment of inflammatory and/or malignant disorders. ■

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RED CELLS

Comment on Ohene-Abuakwa et al, page 838

Diamond-Blackfan anemia: a "cultural" diagnosis

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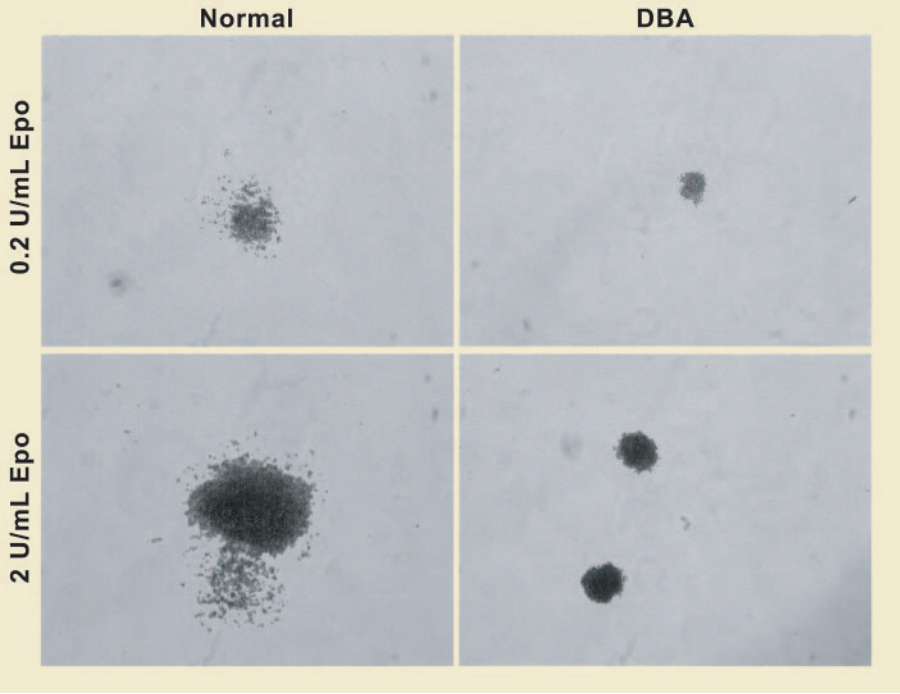
In vitro erythroid cultures may be necessary to detect nonpenetrant Diamond-Blackfan anemia.

The diagnosis of Diamond-Blackfan anemia (DBA) was easy when it was first described in the 1930s: severe congenital anemia in a transfusion-dependent child.[1] DBA is now defined as "[1] normochromic (usually macrocytic but occasionally normocytic) anemia developing early in childhood; [2] reticulocytopenia; [3] normocellular bone marrow with selective deficiency of red cell precursors; [4] normal or slightly decreased leukocyte counts; and [5] normal or often increased platelet counts."[2(p318)] Clinical and molecular genetic studies have revealed that the inheritance of DBA is consistent with an autosomal dominant mechanism; some familial and apparently sporadic cases have germ-line mutations in the *RPS19* gene, which encodes ribosomal protein S19. More complete evaluationsof family members identified individuals who were obligate carriers of DBA by pedigree position and/or documented mutations in *RPS19,* but who clinically did not have the DBA phenotype. Laboratory parameters that support a DBA diagnosis include mild anemia, macrocytosis, elevated fetal hemoglobin (markers of fetallike stress



DBA colonies are smaller than normal at low or high erythropoietin concentrations. Mononuclear cells were cultured in liquid in phase I without erythropoietin, and in semisolid clonogenic culture in phase II. While the numbers of colonies from DBA patients were the same as from normal individuals, the DBA colonies were much smaller, since they contained fewer cells. See the complete figure in the article beginning on page 838.

erythropoiesis),[3] and elevated red cell adenosine deaminase (ADA).[4] However, these findings were not always observed, even in individuals with a definitive "DBA" diagnosis.

Ohene-Abuakwa and colleagues in this issue of *Blood* provide new insights into the pathophysiology of DBA, which also shed light on the silent carrier or nonpenetrant individual. They have adapted the 2-phase liquid erythroid culture system[5] to demonstrate that the pre-erythropoietin stage of erythroid commitment is intact in DBA, while the erythropoietin-dependent phase of erythroid expansion and terminal maturation is defective. They observed this abnormality in late erythropoiesis among DBA patients representing the entire range of phenotypic severity, both with and without detectable mutations in *RPS19*, including nonanemic relatives with increased adenosine deaminase as their only sign of possible DBA.

Confirmation of this "cultural" test as a means of identifying DBA carriers in families without mutations in *RPS19* would lead to a series of important questions that are equally applicable to other dominantly inherited bone marrow failure syndromes in which silent carriers are observed: (1) Will clinical bone marrow failure develop as patients age, despite their normal phenotype? (2) Are such individuals suitable stem cell transplant donors for affected family members? (3) What is the risk of leukemia or solid tumors of the types known to develop in persons with an unequivocal diagnosis of the disorder? (4) What is the risk of clinical bone marrow failure in the offspring of silent carriers? The authors of the current paper mention that anemia developed subsequently in one of the relatives in whom they found reduced in vitro erythropoiesis. There is also a case report of a bone marrow transplant from an HLA-identical sibling in which there was stem cell but not erythroid engraftment,[6] suggesting that there was a specific defect in erythropoiesis.

It is not currently feasible to perform in vitro erythroid culture assays on all relatives in DBA families. Nevertheless, the studies by Ohene-Abuakwa et al suggest that such cultures should be considered in the evaluation of potential stem cell donor relatives, even for those in whom there is no evidence of DBA by hemoglobin, mean cell volume, hemoglobin F, ADA, or a germ-line mutation in *RPS19*. Identification of new DBA genes in *RPS19*-negative families will eventually provide the most direct information regarding affection status, but tissue culture assays of the type described by Ohene-Abuakwa et al may be an important interim approach. Hematopoietic cell biology has not yet been completely replaced by molecular studies. ■

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CLINICAL OBSERVATIONS

Comment on Studt et al, page 542

Hemoglobin versus ADAMTS13

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Free hemoglobin inhibits ADAMTS13 activity in vitro, demonstrating a potential source of artifact affecting ADAMTS13 activity assays and inhibitor screens.

Deficiency of the ADAMTS13 metalloprotease causes thrombotic thrombocytopenic purpura (TTP). The rare familial form of TTP results from mutations in the *ADAMTS13* gene that lead to loss of plasma ADAMTS13 activity.[1] In contrast, the more